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# Polymers for Sustained Macromolecule Release: Procedures to Fabricate Reproducible Delivery Systems and Control Release Kinetics

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Received May 31, 1979, from the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, MA 02139, and the Department of Surgery, Childrens' Hospital Medical Center, Boston, MA 02115. Accepted for publication October 8, 1979.

**Abstract** □ Matrixes composed of ethylene-vinyl acetate copolymer are useful vehicles for the sustained release of macromolecules such as proteins. A new procedure for fabricating these polymeric delivery systems involved mixing the dry, powdered macromolecule with a polymer solution and casting the mixture at -80°. The resulting matrix was dried in two 48-hr stages, first at -20° and then at 20°. These polymer systems had uniform drug distribution, and their release kinetics were reproducible. Fabrication parameters such as drug particle size, drug loading, and matrix coating all significantly affected release kinetics.

**Keyphrases** □ Kinetics, drug release—effects of particle size, drug loading, and matrix coating on polymer-drug mixtures □ Polymers—use in sustained-release film formation, preparation methods of polymer-drug matrix, low temperature effects on polymeric matrix □ Controlled-delivery system—preparation methods, polymer-drug matrix, drug release kinetics

A previous study described the first biocompatible polymeric delivery systems capable of continuously delivering macromolecules (mol. wt. > 1000) such as proteins for prolonged periods (1). In that study, a polymer-macromolecule matrix was formed by mixing powdered macromolecules in a polymer solution. This mixture was poured into small, conical, glass molds, and the solvent was evaporated. The resulting pellets, when exposed to an aqueous medium, released macromolecules in biochemically active form for >100 days (2). Although sustained release was achieved, the reproducibility of the release kinetics was poor. Difficulties in obtaining reproducible release rates were compounded when larger volume polymeric delivery systems were fabricated.

Significant drug settling and redistribution occurred during casting and drying due to the insolubility of the incorporated macromolecules in the polymer solvent. At room temperature, the drug migrated vertically, and visible lateral motion was caused by currents (possibly thermal) in the mixture. The low temperature casting procedures described were developed to minimize this drug movement during matrix formation. The reproducibility of release

kinetics of matrixes prepared using these low temperature methods was improved markedly.

The reproducible kinetics permitted the study of the effects of certain fabrication factors, including drug particle size, drug loading, and matrix coating, on macromolecule release. The significant effects of these factors on release kinetics suggest possible means of utilizing and modifying macromolecular release systems according to research or clinical needs.

## EXPERIMENTAL

**Matrix Preparation**—Ethylene-vinyl acetate copolymer<sup>1</sup> (40% vinyl acetate by weight) was dissolved in methylene chloride<sup>2</sup> to give a 10% solution (w/v). Protein or another macromolecular powder was sieved<sup>3</sup> to give particles of <75, 75–250, or 250–425 μm. A weighed amount of powder from a single size range was added to 15 ml of the polymer solution in a glass vial<sup>4</sup>, and the mixture was vortexed<sup>5</sup> for at least 10 sec to yield a uniform suspension. This mixture was poured quickly into the center of a leveled glass mold (7 × 7 × 0.5 cm), which had been cooled previously on dry ice for 5 min. During precooling, the mold was covered with a glass plate to prevent excess frost formation.

After the mixture was poured, the mold remained on the dry ice for 10 min, and the mixture froze. (The mold was covered again for the last 7 min of this stage.) The frozen slab was easily pried loose with a cold spatula, transferred onto a wire screen<sup>6</sup>, and kept at -20° for 2 days. The slab then was dried for 2 more days at room temperature in a desiccator<sup>7</sup>, under a mild, house-line vacuum (600 mtorr). Drying caused the slabs to shrink to ~5 × 5 cm. The central 3 × 3-cm square was excised with a scalpel<sup>8</sup> and a straight edge and divided further into nine 1 × 1-cm squares.

**Kinetics**—Each square was weighed, its thickness was measured with

<sup>1</sup> Elvax 40, DuPont Chemical Co., Wilmington, Del. This polymer also may be obtained from Dr. Langer at the Massachusetts Institute of Technology.

<sup>2</sup> Fisher Scientific Co., Fair Lawn, N.J.

<sup>3</sup> American Standard sieves, Nos. 40, 60, and 200, Dual Manufacturing Co., Chicago, Ill.

<sup>4</sup> Wheaton Scientific Co., Millville, N.J.

<sup>5</sup> Scientific Industries, Bohemia, N.Y.; set at speed 10.

<sup>6</sup> Common steel mesh, 0.5-mm spacing.

<sup>7</sup> Bel Art, Pequannock, N.J.

<sup>8</sup> No. 10 blade, Bard-Parker, Rutherford, N.J.

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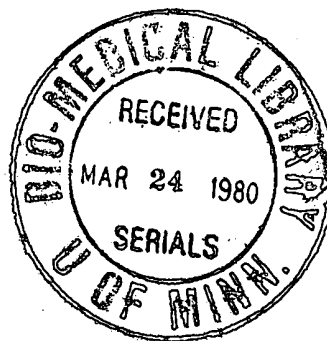
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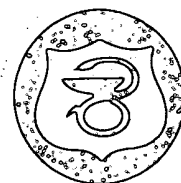
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a micrometer<sup>9</sup>, and it was placed in a vial containing 10 ml of saline (0.154 M NaCl<sup>10</sup>). Vials were put on a shaker<sup>11</sup> at 20°. At each time point, the squares were transferred with forceps into fresh, saline-containing vials. During these transfers, excess solution on the matrix surface was removed by gentle blotting on a tissue. The concentrations of macromolecules in the vials were determined spectrophotometrically<sup>12</sup>.

**Release of Macromolecules**—Kinetic studies were conducted on the release of three proteins<sup>13</sup>: bovine serum albumin (mol. wt. 68,000),  $\beta$ -lactoglobulin (mol. wt. 18,000), and lysozyme (mol. wt. 14,400). A standard particle size (75–250  $\mu$ m) and loading (25% of matrix weight as protein) were used in the measurement of release kinetics. Protein concentrations were determined by absorbance at 220 or 280 nm.

**Particle Size and Loading**—A series of experiments was conducted to compare the release of albumin of three particle sizes (<75, 75–250, and 250–425  $\mu$ m) at four drug loadings (10, 25, 37.5, and 50% protein by weight). With the smallest particle size, a 67% loading was possible and was tested for release kinetics. (With particle sizes greater than 75  $\mu$ m, the polymer solution–protein powder mixture would not pour.)

For each particle size and loading, at least two slabs were cast and assayed for release characteristics. Release from each square was monitored for 4–8 weeks. Table I lists the weights and thicknesses of matrix squares containing albumin of various particle sizes and loadings.

**Isotropic Studies**—Experiments were conducted to examine matrix isotropy by comparing release from the top and bottom faces of polymer matrixes containing albumin. Conditions ranged from 25% loading and particle sizes of <75  $\mu$ m to 50% loading and particle sizes of 250–425  $\mu$ m. For each matrix square, all surfaces except for one face were covered by dripping molten paraffin wax<sup>14</sup> onto the square. The remaining face was protected by adhesive tape. The excess wax was trimmed off, and the adhesive tape was removed.

Release measurements were conducted with care taken to ensure that the unwaxed face always was exposed to the release medium. Release rates were compared between the top and bottom faces of matrixes for each combination of particle size and loading. Control squares containing albumin were covered completely with wax; these squares showed no effect on the spectrophotometric assay.

**Coating**—The effect of coating matrixes with an additional polymer layer was examined. Polymeric matrixes, 50% insulin<sup>15</sup> by weight, were prepared by the described procedures, except that the insulin powder was not sieved. (Commercial insulin powder, with a particle size of <50  $\mu$ m, is more uniform than most protein powder preparations.) After the insulin–polymer matrixes were dried and weighed, the squares were coated by dropping each into a vial containing 15 ml of polymer solution, either 5 or 20% (w/v) ethylene–vinyl acetate in methylene chloride. After 1 min in solution, the square was grasped on its edges with forceps, removed from solution, and held to dry at room temperature for 2 min. The matrix was dried for an additional 24 hr at room temperature under mild vacuum.

Both coated and uncoated samples were measured for release kinetics for over 20 days. Because insulin can bind to glass, release was measured in silicone<sup>15</sup>-coated vials containing saline at 20°. Insulin concentrations were determined by adjusting the pH to 3.0 (50  $\mu$ l of 1 N HCl<sup>10</sup> added/10 ml of saline) and measuring absorbance at 220 nm.

## RESULTS

**Uniformity and Reproducibility**—The improvement in the uniformity of drug distribution using low temperature fabrication methods is shown in Fig. 1. Figure 1a is a photograph of a matrix containing albumin (particle size of 75–250  $\mu$ m, 25% protein by weight), which was cast and dried at low temperature; under these conditions, the drug was distributed uniformly throughout the matrix. Figure 1b shows a matrix similarly prepared, except that casting and drying were conducted at room temperature. In this case, drug distribution was irregular, with some areas nearly void of protein (clear zones).

The low temperature fabrication procedure produced polymer matrixes that were essentially isotropic with respect to release. Experiments in which release rates from the top and bottom faces of the polymer matrix were studied separately showed that release rates never differed by more

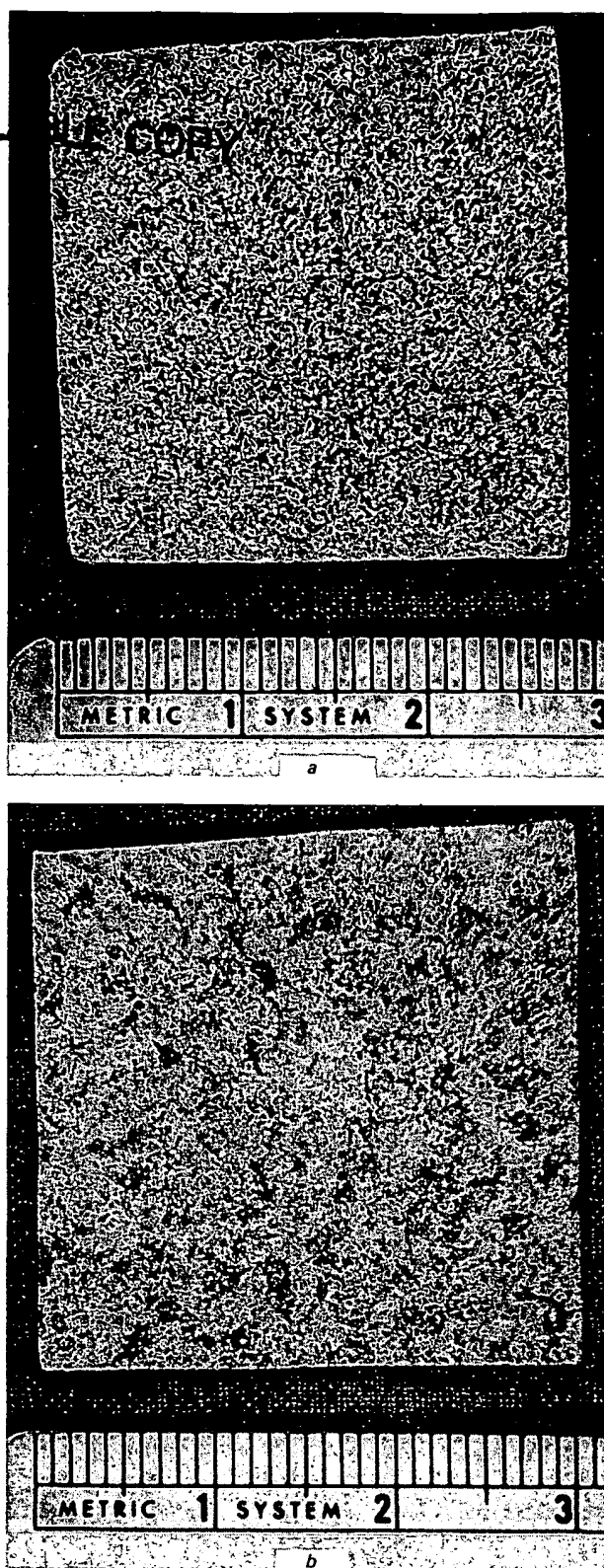


Figure 1—Photographs of matrixes containing bovine serum albumin (particle size of 75–250  $\mu$ m, 25% protein by weight) prepared by the low temperature procedure (Fig. 1a) and at room temperature (Fig. 1b). The scale is in centimeters.

than 25%, which suggests that the low temperatures during the matrix casting and drying prevented significant vertical drug migration.

Figure 2 depicts the reproducibility of release kinetics from matrixes prepared at a low temperature. Three slabs were prepared with albumin, and all 27 1  $\times$  1-cm squares were measured for release for >24 days. The mean daily release rates and their standard deviations are shown in Fig.

<sup>9</sup> Starret Co., Athol, Mass.

<sup>10</sup> USP grade, Mallinckrodt, St. Louis, Mo.

<sup>11</sup> Clinical rotating apparatus, Arthur H. Thomas, Philadelphia, Pa.; set at speed

3.

<sup>12</sup> Gilford Instrument Laboratories, Oberlin, Ohio.

<sup>13</sup> Sigma Chemical Co., St. Louis, Mo.

<sup>14</sup> Gulf Oil Corp., Houston, Tex.

<sup>15</sup> Siliclad, Clay-Adams, Parsippany, N.J.

Table I—Weights and Thicknesses<sup>a</sup> of 1 × 1-cm Matrixes Prepared with Albumin at Various Particle Sizes and Loadings

Loading, % by weight	Particles of <75 $\mu$ m		Particles of 75–250 $\mu$ m		Particles of 250–425 $\mu$ m	
	Weight $\pm$ SD <sup>b</sup> , mg	Thickness $\pm$ Range, mm	Weight $\pm$ SD <sup>b</sup> , mg	Thickness $\pm$ Range, mm	Weight $\pm$ SD <sup>b</sup> , mg	Thickness $\pm$ Range, mm
10	67.3 $\pm$ 3.6	0.75 $\pm$ 0.01	67.3 $\pm$ 3.6	0.75 $\pm$ 0.01	56.3 $\pm$ 2.3	0.81 $\pm$ 0.01
25	76.1 $\pm$ 3.5	0.80 $\pm$ 0.02	76.1 $\pm$ 3.5	0.83 $\pm$ 0.01	62.3 $\pm$ 2.3	1.00 $\pm$ 0.02
37.5	87.3 $\pm$ 3.4	0.94 $\pm$ 0.02	87.3 $\pm$ 3.4	0.93 $\pm$ 0.02	68.9 $\pm$ 4.3	1.23 $\pm$ 0.03
50	98.7 $\pm$ 4.4	1.13 $\pm$ 0.03	74.9 $\pm$ 3.0	1.07 $\pm$ 0.03	74.9 $\pm$ 5.3	1.43 $\pm$ 0.04
67	103.6 $\pm$ 6.3	1.74 $\pm$ 0.04	83.1 $\pm$ 5.2	1.47 $\pm$ 0.04	— <sup>c</sup>	— <sup>c</sup>

<sup>a</sup> All weights and thicknesses were measured before exposure to saline. Hydration increased the volume by ~5%. <sup>b</sup>  $n \geq 8$ . <sup>c</sup> This particular combination of particle size and loading was not prepared.

2. Standard deviations of release rates were within 15% of the respective means, except during the early release period (in which the standard deviations were still within 25%). Standard deviations of release rates from matrixes prepared at room temperature were only within 75%. In addition, more than 90% of the total variance of release for matrixes fabricated at a low temperature was due to variability within a single slab.

Other studies using different proteins, particle sizes, and loadings yielded release reproducibilities consistent with the described observation with one exception: at high loadings ( $\geq 50\%$ ), the center square had a significantly slower release rate than the other eight squares from the same slab. This observation may be because the center is the site of pouring and, therefore, is the last area to be fully cooled.

Figure 3 shows the release of different proteins of the same particle size and loading. In this graph, cumulative percent protein release is plotted versus time<sup>1/2</sup>. The standard deviation of cumulative release was within 10% of the mean in all cases.

**Particle Size and Loading**—Figures 4 and 5 show the effects of drug particle size and loading on release kinetics. The profiles of cumulative release versus time<sup>1/2</sup> first are grouped by common loadings ranging from 10 to 50% (Figs. 4a–4d). These release profiles then are plotted by similar particle size (Figs. 5a–5c). In every case, the standard deviation of cumulative release was within 10% of the mean, and there appeared to be a release period roughly linear with respect to time<sup>1/2</sup>. At higher loadings, this period was almost immediate (Fig. 4d). At lower loadings, there was an initial, nonlinear burst of release lasting about 48 hr, followed by a linear period (Figs. 4a and 4b).

Particle size significantly affected the drug release rates. In almost all cases, an increase in particle size increased the release kinetics. The only exception was at the highest loading; in this case, the difference between release rates of polymer matrixes containing protein in the two larger particle-size ranges was minimal. The effect of particle size was most significant at the intermediate loadings (25 and 37.5%). Here, the slopes of the regions linear with respect to time<sup>1/2</sup> varied as much as sixfold due to a change in particle size.

Increases in drug loading uniformly increased drug release rates (Fig. 5). Not only did total drug release increase, but the cumulative percent of matrix drug released over time increased. As much as a 50-fold increase in the percent released slopes was caused by increasing drug loading from 10 to 50% by weight (Fig. 5a).

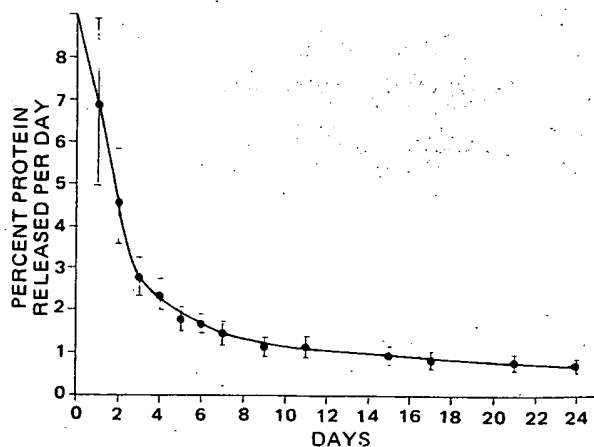


Figure 2—Reproducibility of release rates for 27 matrixes prepared from three slabs. The incorporated protein is bovine serum albumin at a particle size of 75–250  $\mu$ m and 25% loading.

**Coating**—Coating the matrix also significantly affected drug release rates (Fig. 6). Release in coated matrixes again appeared to follow time<sup>1/2</sup> kinetics. Release kinetics decreased with increases in coating solution concentrations. A sevenfold difference in release rates was observed between uncoated samples and samples coated with 20% polymer solution. Standard deviations of cumulative release values were within 15% of the means.

## DISCUSSION

These results show the effectiveness of low temperature fabrication procedures for the incorporation of macromolecules into polymeric matrixes. Procedures used for the incorporation of low molecular weight drugs (3–7) are inadequate for macromolecules. Compression molding and levigation (3, 4) would subject the relatively less stable proteins or macromolecules to denaturation. Therefore, macromolecules were added to a solution of polymer dissolved in a volatile solvent (methylene chloride). This mixture, when cast and dried, produced matrixes capable of sustained macromolecular release.

Sustained-release films for low molecular weight drugs have been cast from polymer solutions in which the drug also was dissolved (5–7). However, macromolecules such as proteins were insoluble in the polymer solution used and rapidly migrated when cast at room temperature. Low temperature casting and drying reduced protein migration and resulted in matrixes with uniform drug distribution and reproducible release kinetics. Similar low temperature techniques may prove useful in other systems that require uniform distribution of an insoluble compound in a polymer solution.

The reproducibility of release kinetics for matrixes prepared by low temperature methods was demonstrated for different proteins and for a range of particle sizes and loadings. However, it may be more difficult to incorporate some macromolecules uniformly if the drug particle density is much greater than that used in the present study since high density may cause the particles to settle significantly even at low temperatures. This settling may be alleviated by more rapid cooling during casting.

The polymer solution mixed with macromolecules quickly gelled when cast on dry ice at  $-80^\circ$ . After 2 days at  $-20^\circ$ , enough solvent had evaporated to allow further drying at room temperature (2 days under mild

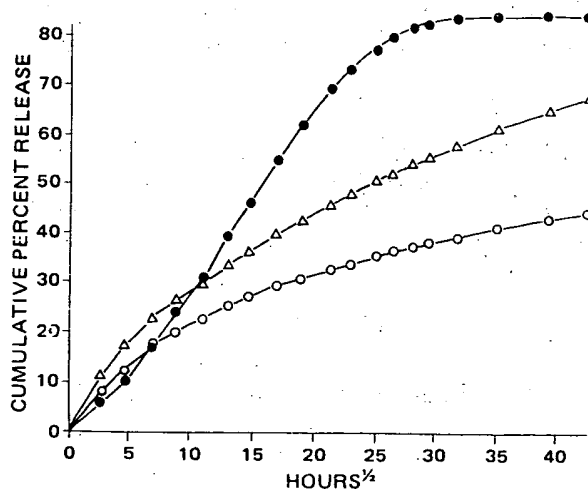
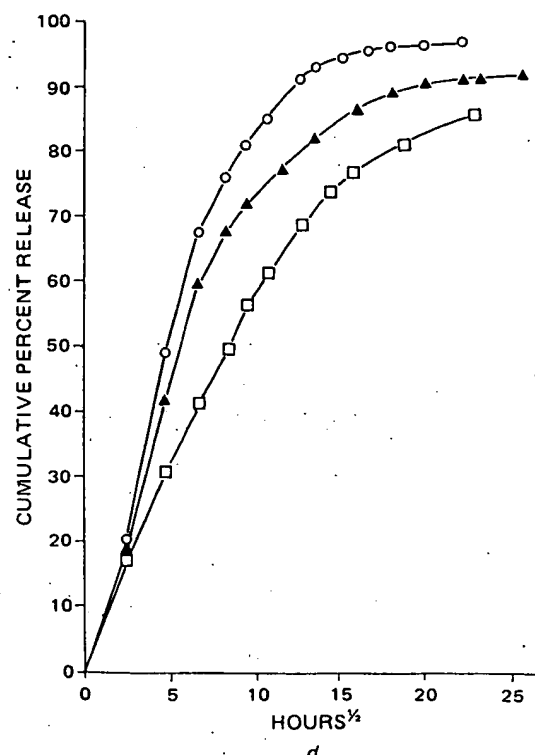
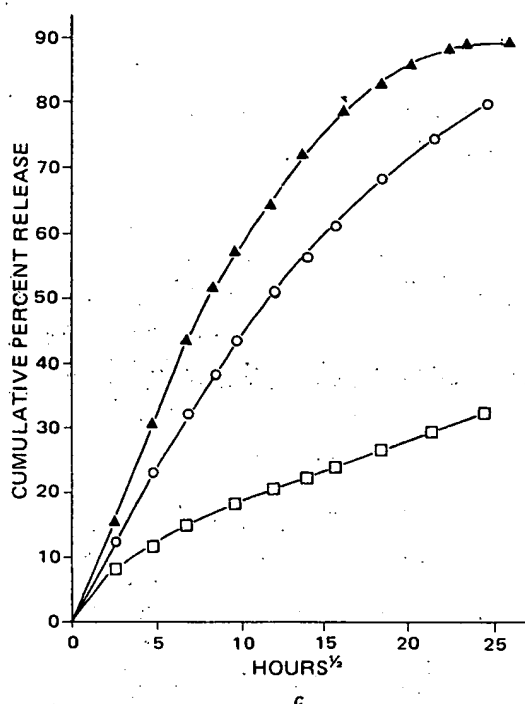
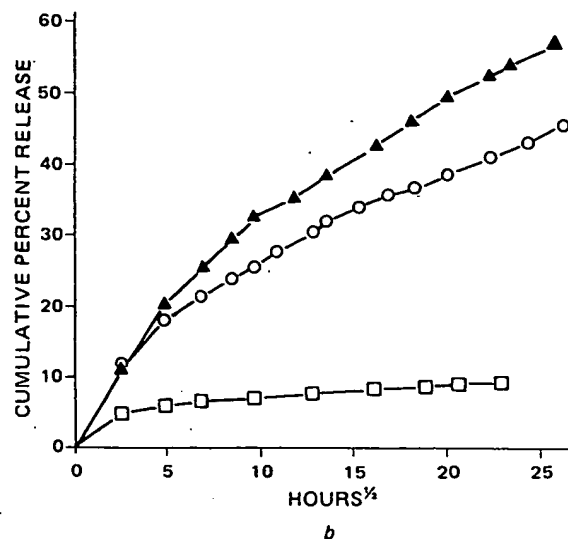
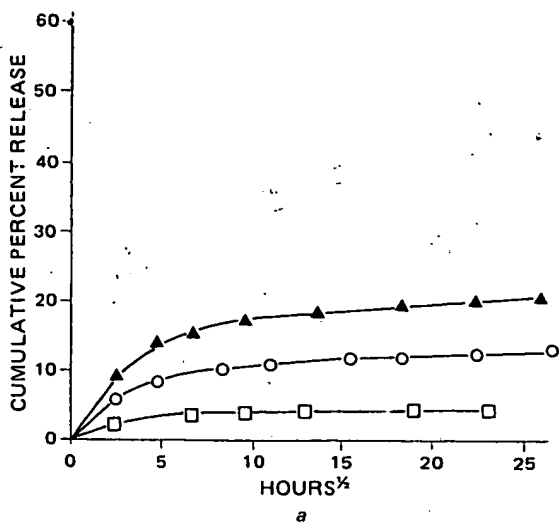


Figure 3—Cumulative release versus time<sup>1/2</sup> for three proteins. Key: ●,  $\beta$ -lactoglobulin;  $\Delta$ , bovine serum albumin; and O, lysozyme. Each point represents the mean of at least eight samples. Standard deviations were <10% of the respective mean values.



**Figure 4—Particle-size effect on cumulative release.** Particles of three size ranges [ $<75$  ( $\square$ ),  $75-250$  ( $\circ$ ), and  $250-425$  ( $\blacktriangle$ )  $\mu\text{m}$ ] were incorporated into matrixes at four loadings (weight percent of albumin per matrix): Fig. 4a, 10%; Fig. 4b, 25%; Fig. 4c, 37.5%; and Fig. 4d, 50%. Each point represents the mean of eight samples. Standard deviations were  $<10\%$  of the respective mean values.

vacuum). In a separate experiment, more extensive drying (up to 60 days under vacuum) did not affect release kinetics.

There appeared to be three release phases: (a) a burst or initial period of rapid release; (b) a period when release was approximately linear with respect to  $\text{time}^{1/2}$ ; and (c) a final period when release tapered off. The burst effect presumably was due to protein dissolution on the surface and cut edges of the matrix. The intermediate release phase that displayed  $\text{time}^{1/2}$  kinetics resembled earlier release models of low molecular weight drugs from granular matrixes (8). Because macromolecules are too large to diffuse through a pure polymer film (1), it is possible that sustained release occurs via diffusion through channels in the matrix. The incorporation of the macromolecules during casting may introduce such channels through which the dissolved drug can diffuse. In this case, observed differences in release rates for different proteins would be attributable to differences in protein properties (solubility and diffusivity) and matrix characteristics (porosity and tortuosity). For example, the very slow release from the uncoated insulin-polymer matrixes (Fig. 6) may result from the small size of the incorporated insulin powder and the low insulin solubility in the release media.

Drug particle size and loading markedly affected release kinetics of the macromolecular polymeric delivery systems (Figs. 4 and 5). Similar observations have been made for low molecular weight drug release (9, 10), although the effects were not as significant. Release rate increases caused by increases in particle size may result from the formation of larger channels or pores in the polymer matrix. Similarly, increased loadings may provide simpler pathways (lower tortuosity) and greater porosity for diffusion, both of which would facilitate the movement of water into, and proteins out of, the matrix.

A coating also can be used to control macromolecular release kinetics. Its effects may be due to the reduced amount of macromolecule on the matrix surface as well as the decreased surface access of pores for channeled diffusion. It is assumed that the coating procedure does not cause a pure intact polymer film to form around the matrix since that would prevent any macromolecular diffusion into the surrounding media (1).

Systems for the sustained release of macromolecules have potential biological and pharmacological value. For example, these systems provided the basis for bioassays of growth factors derived from tumors (2), cartilage (11), the vitreous body (12), and macrophages (13). They also

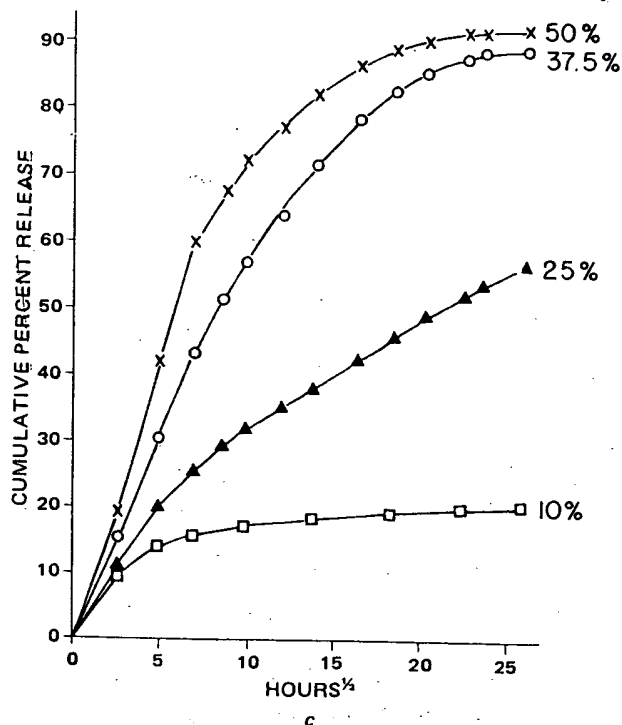
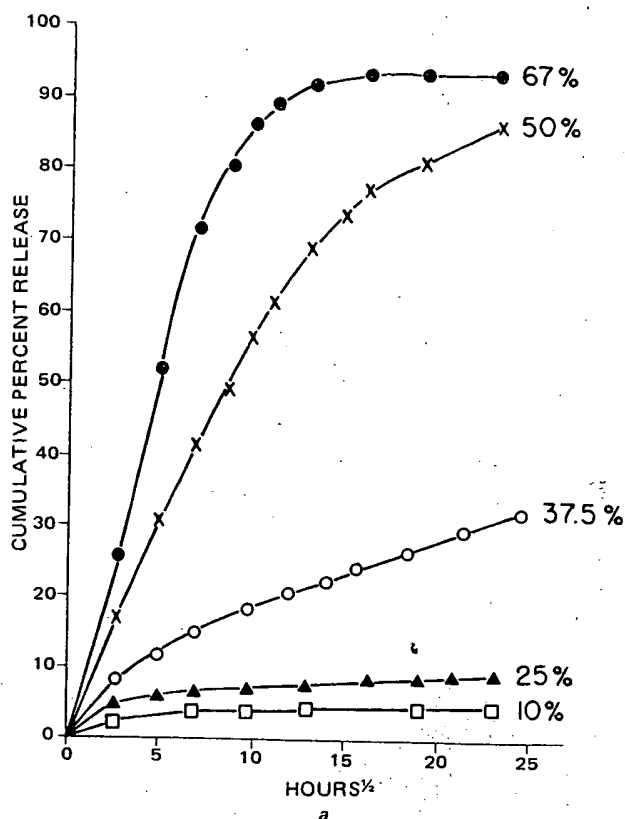


Figure 5—Loading effect on cumulative release. Matrixes with loadings [10 (□), 25 (▲), 37.5 (○), 50 (×), and 67% (●) of albumin by weight] were made using albumin of three particle-size ranges: Fig. 5a, <75 μm; Fig. 5b, 75–250 μm; and Fig. 5c, 250–425 μm. Each point represents the mean of at least eight samples. Standard deviations were <10% of the respective mean values.

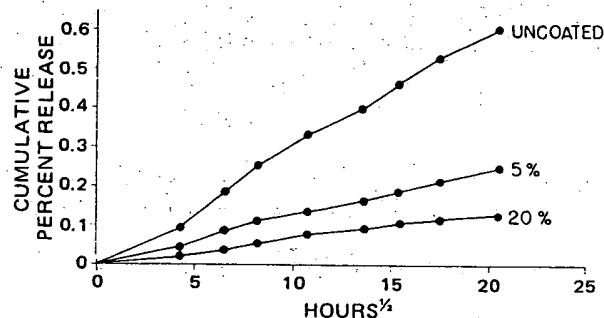
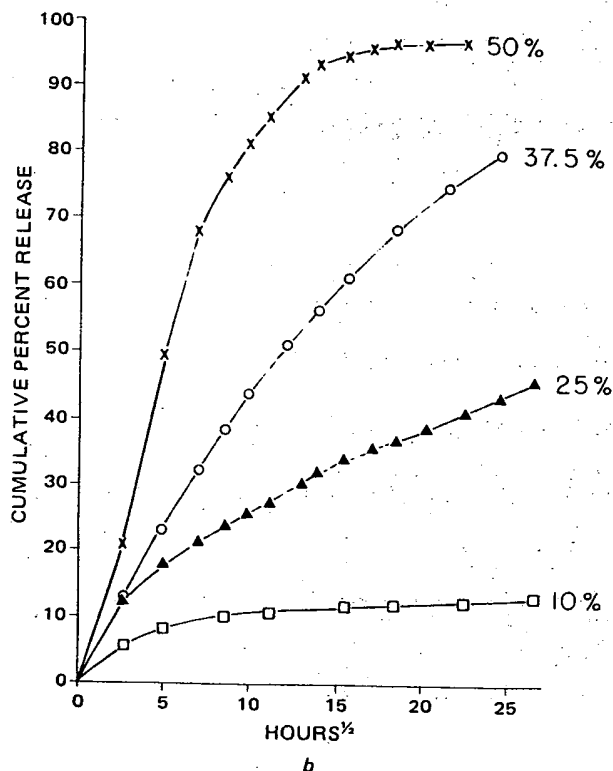


Figure 6—Coating effect on the cumulative release of insulin. Each point represents the mean of eight samples. Standard deviations were <15% of the respective mean values.

being directed toward the development of physical and mathematical models to describe and predict macromolecular release.

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were used in studies of vascular proliferation (14), vascular regression (15), and chemotaxis (16). Clinically, these systems may prove valuable as single-step methods for immunization (17) or for the continuous delivery of insulin (18) and other high molecular weight drugs.

These low temperature fabrication procedures for macromolecular release systems are easy to perform and require standard, inexpensive laboratory apparatus. A wide spectrum of release rates can be achieved by altering drug particle size and loading and by coating the matrix; thus, these systems can be modified for various applications. Further work is



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## Solvent Effects on IR Spectra of Flurazepam

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**Abstract** □ Differences among the IR spectra of flurazepam samples obtained by recrystallizing flurazepam dihydrochloride from different solvents were studied and found to be caused by different mixtures of flurazepam mono- and dihydrochlorides and the free base; these spectral differences are not caused by polymorphism or decomposition of flurazepam.

**Keyphrases** □ Flurazepam—IR spectral analysis, effects of various extraction solvents, transformation from dihydrochloride to monohydrochloride and free base, comparison of spectra of extracted, untreated, and solution samples □ Spectroscopy, IR—flurazepam analysis, effect of various extraction solvents □ Sedatives—flurazepam, IR spectroscopy

To identify the active ingredient in flurazepam dihydrochloride capsules, flurazepam dihydrochloride was extracted from the capsules and its IR spectrum was matched with a reference spectrum. The IR spectrum of the extracted substance in potassium bromide was significantly different from the spectrum of untreated flurazepam dihydrochloride in potassium bromide, as well as from that published previously (1). Different spectra also were obtained in potassium bromide when different extraction solvents were used. The observed differences in the IR spectra were investigated to determine their origin.

#### EXPERIMENTAL

The spectra were recorded by a grating IR spectrophotometer<sup>1</sup>. The samples were measured as potassium bromide disks, as solutions in cells with sodium chloride windows, and as a capillary film between two sodium chloride disks.

The samples were prepared using the following extraction methods.

1. The contents of three 15-mg flurazepam dihydrochloride capsules<sup>2</sup> were extracted with 10 ml of methanol or acetone and filtered. The filtrate was evaporated to dryness under a nitrogen stream, and the residue was collected.
2. Flurazepam dihydrochloride (0.5 g) was dissolved in 3 ml of methanol and evaporated to dryness under nitrogen.
3. Flurazepam dihydrochloride (0.5 g) was dissolved in 100 ml of chloroform with stirring, and the clear solution was evaporated to dryness under nitrogen.

4. Flurazepam dihydrochloride (0.5 g) was dissolved in 2 ml of distilled water and evaporated to dryness under nitrogen. The residue was dried in a vacuum oven at ~50°, if necessary.

5. The contents of two to three flurazepam dihydrochloride capsules (or 100 mg of flurazepam dihydrochloride) were mixed well with 10 ml of distilled water. The mixture was neutralized with 1.0 N NaOH, and the milky solution was extracted with 10 ml of chloroform. The chloroform layer (flurazepam free base) was passed through anhydrous sodium sulfate and evaporated to dryness under nitrogen.

#### RESULTS AND DISCUSSION

The IR spectra of materials (in potassium bromide) extracted from three 15-mg flurazepam dihydrochloride capsules with methanol and acetone are presented in Fig. 1 (curves A and B, respectively). Figure 1 also includes the spectra of untreated flurazepam dihydrochloride, flurazepam monohydrochloride, and flurazepam free base as potassium bromide pellets (curves C, D, and E, respectively). The spectra of the materials extracted with methanol and acetone disagree with each other as well as with the spectra of untreated flurazepam dihydrochloride. At this point, the suggestion of polymorphism arose. The IR spectra (as potassium bromide pellets) of the substances recrystallized by dissolving flurazepam dihydrochloride in methanol, chloroform, and water were obtained (curves A, B, and C, respectively, Fig. 2). Again, these spectra are not identical to one another or to the spectrum of untreated flurazepam dihydrochloride. To investigate whether polymorphism was causing these differences, solution spectra of the same substances were obtained (Fig. 3). Figure 3 also contains the solution spectrum of untreated flurazepam dihydrochloride. Since significant differences were found in the solution spectra as well, these differences could not be attributed to polymorphism.

The differences among curves C-E in Fig. 1 are most pronounced between 1800 and 2500 cm<sup>-1</sup> and at 1635 cm<sup>-1</sup>. The 1800-2500-cm<sup>-1</sup> region contains the absorption bands due to the R<sub>3</sub>N<sup>+</sup>H and R<sub>2</sub>C=N<sup>+</sup>H ions. Saturated tertiary amine salts (R<sub>3</sub>N<sup>+</sup>H) exhibit a strong and broad ammonium band (2) centered between 2300 and 2500 cm<sup>-1</sup>, particularly when a large asymmetric tertiary ammonium ion combines with a relatively small negative ion such as chloride. This band has been observed in many tertiary amine salts of hydrochloric acid and hydrobromic acid.

Unsaturated amine salts (C=N<sup>+</sup>H) exhibit a characteristic immonium band between 1800 and 2000 cm<sup>-1</sup> in addition to the ammonium band between 2300 and 2500 cm<sup>-1</sup>, which overlaps with the ammonium band caused by the R<sub>3</sub>N<sup>+</sup>H ion. The IR spectrum of flurazepam dihydrochloride is characterized by the immonium band between 1800 and 2000 cm<sup>-1</sup> as well as the ammonium band between 2300 and 2500 cm<sup>-1</sup> (curve C, Fig. 1). The IR spectrum of flurazepam monohydrochloride is characterized only by the ammonium band between 2300 and 2500 cm<sup>-1</sup> and the absence of the immonium band between 1800 and 2000 cm<sup>-1</sup> (curve D, Fig. 1). In flurazepam monohydrochloride, where the hydrochloride

<sup>1</sup> Model 283, Perkin-Elmer.

<sup>2</sup> Dalmane, Hoffmann-La Roche.

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